

### ABSTRACT OF THE INVENTION

Loline alkaloids (LA), which are 1-aminopyrrolizidines with an oxygen bridge, are produced by *Epichloë* (anamorph = *Neotyphodium*) species, endophytes of grasses. LA are insecticidal, thus helping protect host plants from insect herbivory. Suppression subtractive hybridization PCR was used to isolate transcripts up-regulated during loline alkaloid production in cultures of *Neotyphodium uncinatum*. Subtracted cDNAs were cloned, and a  $\lambda$ -phage cDNA library from an LA-expressing *N. uncinatum* culture was screened with subtracted cDNA. In BLAST searches, several cDNAs identified had sequence similarities to aspartate kinases, and another with *O*-acetylhomoserine-(thiol)lyase. Differential expression of these two genes in LA-producing cultures of *N. uncinatum* was confirmed, and in a survey of 23 isolates from 21 *Neotyphodium* and *Epichloë* species these two genes strictly correlated with LA production. Two nucleic acid molecules encoding two loline alkaloid gene clusters have been identified.